Express Mail No. EV529814243US

International Application No.: PCT/US2005/008866

International Filing Date: 16 March 2005

Preliminary Amendment

## Amendments to the Specification

Please replace the paragraph beginning at page 3, line 22, with the following redlined paragraph:

Figure 6 shows nuclear translocation of NF-kB in THP-1 cells (monocyte cell line) untreated (from left, first panel, images; second panel, quantitation of first panel images) and treated with LPS (third panel, images; fourth panel, quantitation of third panel images). Images are from darkfield, NF-kB labeled, brightfield, and 7-AAD nuclear label labeled.

Please replace the paragraph beginning at page 4, line 6, with the following redlined paragraph:

Figure 11 shows images of nuclear translocation of NF-κB in adherent A-549 cells untreated (from left, first panel, images; second panel, quantitation of first panel images) and treated with IL-1 $\beta$ /TNF- $\alpha$  (third panel, images; fourth panel, quantitation of third panel images). Images are from darkfield, NF-kB labeled, brightfield, and 7-AAD nuclear labeled.

Please replace the paragraph beginning at page 14, line 16, with the

12 line 27

Change(s) applied to document,

/J.L.W./

12/28/2011

following redlined paragraph:

By way of background and wishing to be bound by theory, NF-κB resides predominantly in the cytoplasm in resting cells. Activating treatments (e.g., IL-1 β /TNF- $\alpha$  or LPS) induce NF- $\kappa$ B translocation into the nucleus in responsive cell types. Thus, the ratio of nuclear to cytoplasmic NFkB-NF-KB increases with LPS treatment. Similar to the A-549 cells, NF-κB is translocated from the cytoplasm to the nucleus when the non-adherent human monocyte cell line, THP-1, is exposed to lipopolysaccharide (LPS). Using the identical probing protocol and CCF, again a quantifiable difference in the nuclear localization NF-kB is demonstrated when comparing untreated and LPS-

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treated cells (*see* Figures 6 and 9). A nuclear and NF-κB pixel signal correlation analysis CCF was used to quantitate the difference between untranslocated NF-κB and NF-κB translocated to the cell nucleus. The CCF distinguished location-specific (nuclear and cytoplasmic) quantitation of NF-κB to distinguish LPS-treated from untreated THP-1 cells. Thus, the methods of the present disclosure may also be used with non-adherent cells and cell lines.

Change(s) applie to document, /J.L.W./ 12/28/2011	ed redlined section			replace the section beginning at page 17, line 1, with the following
	A.	Materi	als	
			01.	anti-NFkB (F6): Santa Cruz Biotechnology (Cat. No.SC-8008),
	200 μg/ml			
			02.	Alexa Fluor488 donkey anti-mouse IgG: Molecular Probes (Cat.),
	1.1 mg	g/ml		
			03.	Streptavidin Alexa Fluor 488: Molecular Probes
			04.	Recombinant human TNF-α: BD (Cat# 554618, Lot#
	0000056653)			
			05.	Recombinant human IL-1 : ebi0science eBioscience (Cat# 14-
	8018-6	52 <del>, Lot#</del>	<del>2</del> )	
			06.	A549 cells (ATCC No. CCL-185)
			07.	Dulbecco's MEM
			08.	Fetal Calf Serum
			09.	F-25 Culture Flask
			10.	0.25 % trypsin / EDTA
			11.	Phosphate buffered saline without Ca <sup>2+</sup> /Mg <sup>2+</sup> (PBS)
			12.	4% PFA/PBS (Fixation Buffer)
			13.	0.1% triton X-100/PBS (Perm Buffer)